



FEDERAL SECURITY AGENCY
U. S. PUBLIC HEALTH SERVICE

IN REPLYING, ADDRESS THE

July 20, 1949

Dear Josh,

In our first recombination expt. to your strains,

58-161 & W-677 were grown with a heavy inoc. (1 ml. of an overnight culture of each separately + together) in 5 ml. of minimal medium + 0.2% yeast extract (Difco) + 0.2% casein hydrolysate (Sheffield N2-case) + 50 mT/ml. biotin. After 6 hours of cultivation at 35°C. + shaking, the cells were washed in the manner described in your paper, and 10^{-0} , 10^{-1} , + 10^{-2} ml. of the heavily turbid suspensions plated in 10 ml. of our minimal agar (CPNAS) on top of 15 ml. of same. The results obtained were as follows (vertical columns of successive colony counts at 1, 2, 3, + 4 days).

58-161 $10^{-0}, 10^{-1}, 10^{-2}$	W-677 $10^{-0}, 10^{-1}, 10^{-2}$	Inoc. of each from separate tube			Mixed cult. in tube		
		10^{-0}	10^{-1}	10^{-2}	10^{-0}	10^{-1}	10^{-2}
0	0	0	0	0	>1000	12	0
0	0	0	0	0	"	28	1
0	0	4	0	0	4	?	4
0	0	4	1	0	(colonies mostly, mixed)	197	4

The irregularity that disturbed us in this expt. is the large drop from 10^{-0} to 10^{-1} inoc. In a second expt. the results were smoother; in this we used a medium with 0.5% YE + 50 mT/ml biotin, but no NZ. At 48 hrs. incub. of the plates, the plate mixture gave 4 cols. from 10^{-0} of each of the two strains, 1 col. for 10^{-1} of each; whereas the tube mixture (4 hrs. growth) gave 141 from 10^{-0} , 55 from 3×10^{-1} , 17 from 10^{-1} , + 8 from 10^{-2} . These were all smooth; on further incubation, smaller non-mixed colonies continued to appear.

In another expt. which used your mutants with the same requirements but without the superposed heterozygosity for virus + fermentations (I don't have the sheet here - I think it was Y-10 instead of one of the above),

the prototropes obtained were not uncoordinated, but the results were otherwise much the same - very few prototropes from plate mixtures, perhaps 50x as many from tube mixtures grown a few hours.

I see no obvious reason why this phenomenon should have turned up in our experiments but not in yours - tho there's nothing special about our minimal medium, I suppose you'll have to try it if you want to duplicate our conditions. The other factors seem pretty standard.

If you feel this presents a serious problem, I'll be glad in September to set up any parallel experiments that you believe would help clarify it.

I'm glad Gordon Allen is definitely going to your lab at the beginning of August - I'm sure he'll learn a lot, & I hope you enjoy the visit.

Sincerely,

T. J. Ewer